Most importantly, the narrative does not include discussion of critical uncertainties in relying on the mouse data from NTP (1998) to predict the potential for carcinogenic risk in the humans, given ample evidence of important pharmacokinetic differences between mice and other species. In fact, the NTP study and other animal studies show that there is little evidence of consistent tumorgenicity across species other than the mouse and in particular the hamster (see Section 3). This difference can clearly be explained by evidence of differences in the pharmacokinetics of chloroprene across species. In addition, consideration of the lack of evidence of the carcinogenicity of chloroprene from human studies and the risks that would be predicted relying on the results from human studies (see Section 11) further indicate that a classification of "likely" carcinogen is inappropriate.

The weight of evidence supports a reclassification. According to US EPA (2015) the updated classification narrative should address the following:

- The weight of the evidence should be presented as a narrative laying out the complexity of information that is essential to understanding the hazard and its dependence on the quality, quantity, and type(s) of data available, as well as the circumstances of exposure or the traits of an exposed population that may be required for expression of cancer.
- In borderline cases, the narrative explains the case for choosing one descriptor and discusses the arguments for considering but not choosing another.
- The descriptors can be used as an introduction to the weight of evidence narrative. The complete weight of evidence narrative, rather than the descriptor alone, provides the conclusions and the basis for them.

A complete and accurate narrative also should capture and interpret all documented major uncertainties in the evidence as it relates to the classification of chloroprene. Transparent documentation of methods, data and assumptions, coupled with an accurate and informative classification of the weight of evidence is needed. Considering the misinterpretation of some data and the uncertainty in relying on responses in the mouse to be predictive of the potential for carcinogenicity in humans, the current classification of "likely to be carcinogenic to humans" unduly raises public health concerns. We conclude that a descriptor of "suggestive to be carcinogenic to humans" is more representative of the weight of evidence and uncertainties associated with relying significantly on results from a species for which there is evidence of differences that explain the observed sensitivity compared to the human.

7 US EPA DERIVATION OF THE CHLOROPRENE IUR

As described in Section 3, US EPA relied primarily on the findings of a two-year inhalation study conducted by the NTP (1998) in B6C3F1 mice and F344/N rats. Trochimowicz *et al.* (1998) also conducted studies in Wistar rats and Syrian hamsters. The results of the NTP (1998) and Trochimowicz *et al.* (1998) studies showed that the mouse is the most sensitive species to chloroprene among the species tested. US EPA selected the results from the female mouse to be the basis for deriving the chloroprene IUR. However, given the differences in response in the mouse compared to other laboratory species, US EPA should have considered the potential for differences in pharmacokinetics to better characterize and explain the cross-species differences. Although this source of bias is likely the largest and most significant, US EPA applied a number of additional assumptions in deriving the chloroprene IUR that lead to conservative bias and unsupported uncertainty in the IUR. The following sections highlight these key sources of uncertainty.

7.1 US EPA's dose-response modeling applied overly conservative methodology

US EPA determined the point of departure (POD)[5] using dose-response modeling to derive the IUR. Specifically, US EPA estimated the effective dose at a specified level of response (a benchmark dose concentration associated with a 10% risk level [BMD_{10}]) and its lower-bound based on the lower 95% confidence interval of the BMD_{10} ($BMDL_{10}$) for each chloroprene-induced tumor type in the mouse. Having determined that chloroprene was more potent in inducing tumors in mice than in rats, US EPA did not consider the rat data further in developing the IUR. US EPA further noted that the observed differences may be due to species differences in metabolism.

US EPA modeled each mouse tumor endpoint reported in NTP (1998) separately using the US EPA multistage Weibull time-to-tumor model. The multistage Weibull model has the following form:

$$P(d,t) = 1 - \exp[-(b_0 + b_1 d + b_2 d^2 + \ldots + b_k d^k) \times (t - t_0)^c]$$

where P(d,t) represents the lifetime risk (probability) of cancer at dose d (the human equivalent exposure in this case) at time t (a human lifetime in this case); parameters $b_i \geq 0$, for I = 0, 1, ..., k; t is the time at which the animal's tumor status, either no tumor, tumor, or unknown (missing or autolyzed) was observed; t_0 is the latency of response; and c is a parameter which characterizes the change in response with age. For the analysis performed in the 2010 Review, the latency (t_0) was set to zero for all models. The power term parameter c is normally a parameter that is estimated by the BMD software. For some tumors, the model software was unable to calculate this parameter and US EPA had to estimate this value (*e.g.*, for forestomach tumors).

In the modeling, US EPA conservatively considered all tumor types, both benign and malignant. US EPA also assumed that the dose-response was linear in the low

[5] A POD is defined as the point on a dose-response curve that marks the beginning of a low-dose extrapolation. This point is typically a lower bound, expressed in human-equivalent terms, near the lower end of the observed range. This POD is used to extrapolate to lower exposures to the extent necessary.

dose range, based on the assumption that chloroprene has a mutagenic MOA. This approach is not justified by the available scientific evidence; therefore, the assumption of linearity inappropriately adds another level of uncertainty to the IUR.

7.2 Extrapolation from animals to humans should have included use of a PBPK model

In the 2010 Review, US EPA did not use a PBPK model for chloroprene to adjust for differences across species, even though a model was available. At the time, US EPA stated that it did not have sufficient data to validate the model. However, all of the quantitative data necessary to refine and verify the critical metabolic parameters for the existing peer-reviewed model for chloroprene (*i.e.*, Himmelstein *et al*. 2004b) were available and could have been applied to adjust the IUR. Further, since the release of the 2010 Review, additional peer-reviewed studies have been published, demonstrating consistent results and validating the use of the model for dose-response modeling and determination of an appropriate human equivalent concentration for the human IUR (Yang *et al*. 2012, Thomas *et al*. 2013, Allen *et al*. 2014).

Instead of using a PBPK model to account for differences between humans and animals, US EPA used a default approach that entails applying a dosimetry adjustment factor (DAF) that accounts for some differences in the blood:air partitioning in animals compared to humans. US EPA used a DAF of 1.0 (essentially assuming equivalence), based on the unsubstantiated assumption that all the lung tumors observed were the result of systemic effects from chloroprene exposures. US EPA provided no evidence to support the assumption that tumors in the lungs of mice are the result of systemic effects, rather than the more plausible portal-of-entry effects that would result from direct contact of chloroprene with lung tissue.[6] As noted by US EPA (2010a), "treating lung tumors as systemic effects returns the highest composite unit risk (approximately 60% greater than if lung tumors are treated as portal-of-entry effects)."

7.3 Deriving a composite IUR based on multiple tumors is not scientifically supported

Another source of overly-conservative bias in the derivation of the IUR is the use of a composite value of multiple tumor types instead of the standard approach of using the most sensitive species, gender, and endpoint(s). The use of the composite value for chloroprene is not valid. While US EPA assumed statistical independence of different tumor types based on a hypothesized MOA for chloroprene involving the production of epoxide metabolites, the underlying data do not demonstrate mechanistic or biological independence. The mechanism of action in multiple tissues could also be due to dependent events; for example, a liver tumor could be dependent on the generation of the same metabolite as that needed for the development of a lung tumor. Figure 7.1 illustrates how US EPA's assumption of adding risk across multiple tumor sites overestimates the potential overall cancer risk. Figure 7.1 also shows the considerable non-random distribution

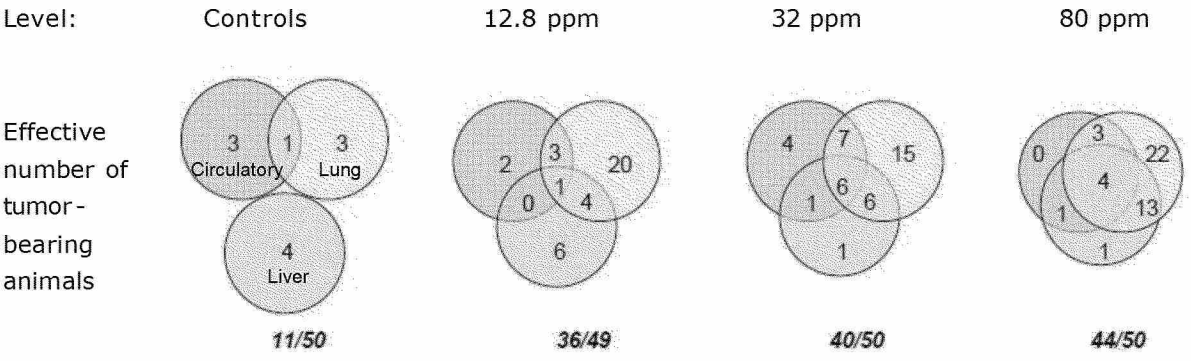
[6] A portal-of-entry effect is a localized effect that occurs at the point at which a substance enters the body (*e.g.*, via inhalation there would be effects on the respiratory system). Systemic effects, on the other hand, are effects that occur in other organs of the body distant from the portal-of-entry (*e.g.*, effects on the liver following inhalation of the substance).

of tumors in the animals bearing multiple tumors . Therefore, when US EPA assumed independence based on an unknown MOA, this inflated the effective number of animals developing tumors and overstated the carcinogenicity of chloroprene. US EPA recognized that the assumption of independence could not be verified, and that if this assumption did not hold, it indeed would overestimate risk (US EPA 2010a), in this case by another 50%.

In calculating the composite estimated IUR, US EPA also assumed that the IURs were normally distributed around the mean with a 95% upper confidence limit that represents the composite estimate. However, there is no evidence to support a normality assumption either in the benchmark dose (BMD) or the IUR, which adds to the uncertainty in the risk estimate.

Based on the US EPA approach of summing IURs for individual tumor types, the estimated composite inhalation IUR for female mice (which were more sensitive to chloroprene than male mice) was increased by approximately 50%, from 1.8×10^{-4} for the most sensitive endpoint (lung tumors in female mice) to 2.7×10^{-4} per $\mu g/m^3$ for all tumors combined. US EPA rounded this to a single significant figure, resulting in an even more conservative IUR for continuous lifetime exposures to adult humans of 3×10^{-4} per $\mu g/m^3$.

NTP Data



US EPA Approach

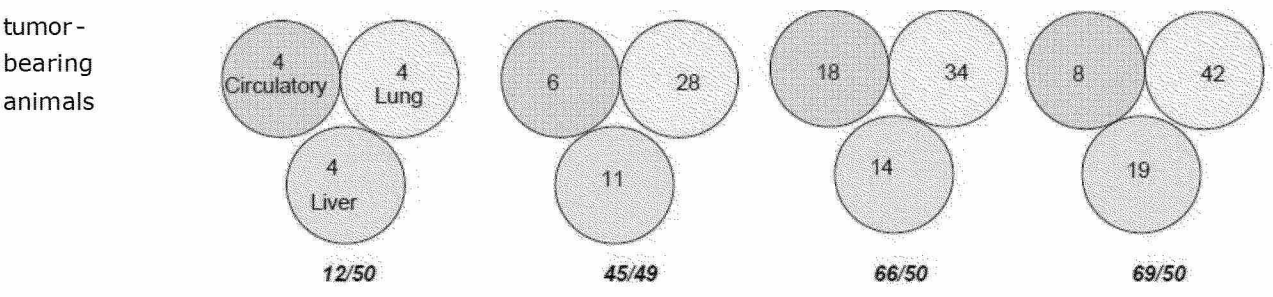


Figure 7.1. Illustration of How US EPA's Approach of Summing Individual Tumor Potencies Overestimates Total Tumor Potency in Female Mice by Assuming Independence.

7.4 IUR adjustment for early life susceptibility is not appropriate

In the final step, US EPA applied an age-dependent adjustment factor (ADAF) to account for early-life susceptibility, because of a hypothesized mutagenic MOA. This yielded a final adjusted unit cancer risk of 5×10^{-4} per µg/m³. This adjustment reflects the use of several sensitivity adjustments for different life-stages, which are applied for presumed mutagenic compounds as specified in US EPA's "Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to Carcinogens" (US EPA 2005). Specifically, as described in the US EPA (2005 b) guidance, US EPA applied the default ADAFs and their age groupings of 10 for <2 years, 3 for 2 to <16 years, and 1 for 16 years and above. The calculations are shown below.

Risk for birth through <2 yr $= 3 \times 10^{-4}$ per µg/m^3 $\times$ 10 $\times$ 2 yr/70 yr $= 8.6 \times 10^{-5}$ per µg/m^3

Risk for ages 2 through <16 $= 3 \times 10^{-4}$ per µg/m^3 $\times$ 3 $\times$ 14 yr/70 yr $= 1.8 \times 10^{-4}$ per µg/m^3

Risk for ages 16 until 70 $= 3 \times 10^{-4}$ per µg/m^3 $\times$ 1 $\times$ 54 yr/70 yr $= 2.3 \times 10^{-4}$ per µg/m^3

The individual risk estimates were then summed to obtain the final lifetime (70 years) IUR for chloroprene:

$$\text{Risk} = 8.6 \times 10^{-5} + 1.8 \times 10^{-4} + 2.3 \times 10^{-4} = 5.0 \times 10^{-4} \text{ per µg/m}^3$$

As with the calculation of a composite IUR (which was increased by 67% based on the combination of tumors), US EPA's assumption of a mutagenic MOA increased the calculated IUR by another 67%. Taken together, these assumption s increased the IUR calculation to 178% of the IUR calculated based on the most sensitive species at the most sensitive site. As discussed in detail in Section 4, the ADAF adjustment is not applicable to chloroprene because there is insufficient evidence of a mutagenic MOA for chloroprene.

7.5 Summary of US EPA's derivation of the chloroprene IUR

The chloroprene IUR derived in the 2010 Review was based on the following assumptions, some of which are not scientifically substantiated:

1. US EPA selected the most sensitive species, female B6C3F1 mice, based on the results from the NTP (1998) study;
2. US EPA assumed lung tumors in mice to be a systemic lesion and not a portal-of-entry effect, resulting in a minimal dosimetric adjustment for extrapolating from animals to humans (i.e., application of a DAF =1);
3. US EPA calculated a composite risk estimate based on multiple tumor sites, although multi-tumor data were inconsistent and relatively weak for most tumor sites;
4. US EPA rounded the IUR prior to applying the ADAF, increasing the IUR further; and
5. US EPA applied an ADAF based on the assumption of a mutagenic MOA.

Table 7.1. Conservative Assumptions in the Calculation of the Chloroprene IUR

Step	IUR per $\mu g/m^3$	Basis	Amount of overestimate	Cumulative overestimate
Most sensitive endpoint/species (portal -of-entry DAF=1.7)	1.06×10^{-4}	Lung tumors in female mice as a portal-of-entry effect		
Most sensitive endpoint/species (systemic lesion DAF=1)	1.8×10^{-4}	Lung tumors in female mice as a systemic effect	1.7	
Multiple tumor adjustment	2.7×10^{-4}	Multiple tumors	1.5	
Rounding	3×10^{-4}	Rounding	1.1	2.8
Application of ADAF	4.5×10^{-4}	Adjustment (without rounding)	1.5	4.2
Application of ADAF	**5×10^{-4}**	**Adjustment (with rounding)**	**1.7**	**4.8**

Combined, these assumptions contribute to a risk estimate that is over-estimated by about a factor of 5 (Table 7.1). However, these assumptions contribute only to a small overestimate compared to consideration of the documented differences across species, which was reported by Allen *et al.* (2014) and confirmed by our own calculations of an updated IUR. Consideration of pharmacokinetic differences across species indicate that the chloroprene IUR is likely overestimated by two orders of magnitude.

7.6 Replication of US EPA's dose-response modeling

The 2010 Review used the results from the NTP (1998) study in mice to calculate multiple PODs for derivation of the composite IUR (see previous section). US EPA focused specifically on the female mouse as this was the most sensitive species and gender, but assumed that this animal model was directly applicable to humans. Further, US EPA assumed a default linear dose-response and applied the multistage Weibull model, which accounts for the influence of competing risks (such as early death) and for the occurrence of multiple tumors, some of which are incidental (benign or not fatal), and others which are carcinogenic (*i.e.*, fatal).

Ramboll Environ attempted to re-create the dose-response modeling for the female mouse endpoints using the same time-to-tumor model provided in the current version of the US EPA BMD software. However, we could not completely replicate US EPA numbers. In attempting to do so, we identified several inconsistencies in the US EPA method and other issues that prevented full replication of US EPA's estimates. Furthermore, we were unable to identify adequate documentation supporting US EPA's calculations. The need for transparency highlighted by the NRC (2014), and as underscored by our inability to replicate the 2010 IUR, demonstrate the need to review and revise the IUR for chloroprene.

Examples of the inconsistencies encountered in our independent modeling of the NTP (1998) data included the following:

1. We were unable to confirm which version of the US EPA Benchmark Dose Modeling Software was used to conduct the modeling presented in the 2010 Review. This is significant because it appears that US EPA used a version of the model (from 2009) that may have contained important errors that were later corrected (personal communication with John Fox, US EPA, June 16, 2016). This could also explain some of the discrepancies in our results compared to those presented in the 2010 Review.
2. US EPA did not provide the complete input files for the model, but only a summary; therefore, we could not verify the data needed for conducting the time-to-tumor model (time of death of the animals, tumor status: censored (C) for no tumor, incidental (I) or fatal (F) tumors, or unknown (U) when there is no tissue or tissue was unusable). The lack of transparency made it difficult to verify whether US EPA conducted the modeling appropriately.
3. For the analysis of the incidence of forestomach tumors, US EPA calculated a power parameter (c), as described above, outside of the modeling program and entered it as a specific variable in the analysis. This parameter necessarily was calculated outside of the program because the program was unable to calculate it. It was unclear how US EPA calculated this parameter and whether this value is larger or smaller than what would be predicted by the program. This could impact the results and introduced additional uncertainty.
4. US EPA did not apply a consistent methodology across all the endpoints and time points that were examined. For example, in some cases animals that had no tumors or evidence that tumors were naturally "digested" by the animal (autolyzed tumors) were simply removed from the analysis (*e.g.*, for the forestomach analysis) and in other cases these were treated as "unknown" tumors (*e.g.*, in the mammary analysis). This approach would result in an overestimate of risk and there was no clear reason why US EPA took this approach.
5. There were also inconsistencies in the number of animals that were reported in each endpoint and time-point group. For example, the number of animals considered in Table C-1 of the 2010 Review (data from NTP 1998) did not match the numbers in Table 5-4 (US EPA 2010a). The major differences were identified in the total number of animals examined for tumors of the skin, mammary gland, forestomach, Harderian gland, and Zymbal's gland, and for the dose levels up to 32 ppm, depending on the endpoint. US EPA reported that tissue from 50 animals was examined, whereas NTP (1998) reported that tissue from only 49 animals was examined. Although this may not have impacted the results significantly, it indicated that US EPA allowed errors in their reporting of the results and possibly made errors inputting the results into the model, some of which might be consequential. Without full transparency and availability of model inputs, this could not be verified.

Ramboll Environ analyzed each endpoint independently, as was done by US EPA, but did not combine the estimates to obtain a composite IUR. We did not agree that US EPA's approach was standard or scientifically justified given that independence could not be confirmed and the MOA across tumor types was unknown. In addition, we corrected the issues associated with the appropriate counts and, following US EPA guidance, removed any unknowns when using an incidence-only analysis (assuming all tumors observed were incidental and were not fatal to the animals). A comparison of our independent results and those generated by US EPA is presented in Table 7.2.

Table 7.2. Comparison of Dose-Response Modeling for Female Mice at a Benchmark Response of 0.01

Site	US EPA Results from Tables C-3 and C-4							Ramboll Environ Results							
	Stage	LL	χ2	AIC	Model Selection	BMD ppm	BMDL ppm	Stage	LL	χ2	p-value	AIC	Model Selection	BMD ppm	BMDL ppm
Lung					One-stage model			3	-83.0	-0.11	0.74	176.04			
								2	-82.96	0.00	1.00	173.93			
	1	-83.02	—	172.0		0.11	0.09	1	-82.96			171.93	Lowest AIC	0.11	0.08
Hemangiomas, hemangio-sarcomas, (fatal) (highest dose group dropped)	3							3	FAILED			279.74			
	2	-135.85	5.34	279.7	χ2, lowest AIC	3.12	0.64	2	-135.87	5.34	0.02	279.74	Lowest AIC	3.04	0.47
	1	-138.52	—	283.0				1	-138.54			283.08			
Hemangiomas, hemangio-sarcomas, (all incidental) (highest dose group dropped)	3							3	FAILED						
	2	-65.81	2.28	139.6	Lowest AIC	4.61	2.02	2	-65.74	2.22	0.14	139.48	Lowest AIC	4.60	1.92
	1	-66.95	—	139.9				1	-66.85			139.70			
Harderian gland	3	-58.26	0.02	126.5				3	-58.22	0.02	0.89	126.45			
	2	-8.27	0	124.5				2	-58.23	0.00	0.98	124.47			
	1	-58.27	—	122.5	Lowest AIC	2.58	1.20	1	-58.23			122.47	Lowest AIC	2.50	1.14
Mammary gland carcinomas, adenoacanthomas	3				One-stage model			3	-84.21	0.00	1.00	178.42			
	2							2	-84.21	0.00	0.99	176.42			
	1	-87.96	—	181.9		1.95	1.34	1	-84.21			174.42	Lowest AIC	2.03	1.38
Forestomach	3	-19.17	0.84	48.35				3	-19.18	0.84	0.36	46.36			
	2	19.60	2.35	45.19	Lowest AIC	20.94	5.69	2	-19.60	2.35	0.13	45.20	Lowest AIC	20.54	5.48
	1	-20.77	—	45.54				1	-20.78			45.55			
Hepatocellular adenomas, carcinomas	3				One-stage model			3	-119.94	0.00	1.00	249.87			
	2							2	-119.94	0.00	1.00	247.87			
	1	-119.2	—	245		0.40	0.23	1	-119.94			245.87	Lowest AIC	0.39	0.23
Skin	3				One-stage model			3	-87.395	0.00	1.00	184.79			
	2							2	-87.395	0.00	0.99	182.79			
	1	-87.463	—	180.9		0.91	0.67	1	-87.395			180.79	Lowest AIC	0.89	0.67
Zymbal's gland	3	-11.402	0.65	32.8				3	-11.406	0.66	0.42	32.81			
	2	-11.726	1.77	31.45				2	-11.734	1.76	0.19	31.47			
	1	-12.611	—	31.22	Lowest AIC	15.78	5.76	1	-12.612			31.22	Lowest AIC	29.9	8.23

AIC: Akaike Information Criterion; BMD: benchmark dose; BMDL: lower 95% confidence limit of the benchmark dose; LL: log likelihood

7.7 Conclusions

US EPA applied a number of scientifically unsupported conservative assumptions in deriving the IUR for chloroprene that resulted in substantial overestimation of the IUR and added uncertainty to the toxicity estimate. Consistent with the majority of available IRIS profiles on other chemicals, the IUR should be based on the most sensitive endpoint in the most sensitive species, as this will be protective for other effects. Not assuming a systemic lesion for lung cancers yields an initial IUR of 1.06×10^{-4} based on the female mouse as the most sensitive species. In recommending a final IUR based on the mouse data, US EPA should have considered the significant pharmacokinetic differences between species and applied the PBPK model for extrapolating from animals to humans (Himmelstein *et al*. 2004), as demonstrated in Section 10.

8 THE CHLOROPRENE IUR COMPARED TO KNOWN CHEMICAL CARCINOGENS

The chloroprene IUR reported in the 2010 Review is much higher than those of similar chemicals, including known carcinogens. We compared (and summarize below) the IURs for all compounds classified by IARC as Group 1 (carcinogenic) or 2A (probably carcinogenic), which generally correspond with US EPA's classification for known or likely/probable human carcinogens. We used IARC classifications because IARC generally applied consistent methods and criteria for evaluating human carcinogens.

We also obtained the US EPA WOE classification and basis of the IUR for carcinogens for which US EPA has calculated and reported an IUR. These compounds are summarized in a table developed and updated by US EPA to be used in dose-response assessments of hazardous air pollutants.[7] In the US EPA table, all hazardous air pollutants are listed with available toxicity values based on source.

We excluded metallic compounds, which tend to be associated with particulate exposures, and mixtures, such as coke oven emissions. We sorted the remaining compounds by the IUR calculated by US EPA, from highest to lowest (Table 8.1). In addition, the table shows the WOE conclusions by IARC, the dates of each evaluation, and the relative strength of the epidemiological evidence. More detailed information on the toxicity evaluations and epidemiological evidence can be found in Appendices A and B, respectively.

[7] See Table 1 available at https://www.epa.gov/fera/prioritization-data-sources-chronic-exposure

Table 8.1. Summary of Potentially Carcinogenic Compounds by IUR Listed in IRIS

Chemical Name	US EPA WOE	Year	IARC WOE	Year	IUR per µg/m3	MOA	Basis of IUR/ Endpoint	Strength of Epidemiology Evidence
Benzidine	A	1987	1	2012	0.067	M*	Human/ bladder	Moderate
Bis(chloromethyl) Ether (BCME)	A	1988	1	2012	0.062		Rat/lung	Moderate
Nitrosodimethyl - amine (NDMA)	B2	1987	2A	1987	0.014	M*	Rat/liver	Limited
Ethylene dibromide	LH	2004	2A	1999	0.0006		Mouse/ nasal	Limited
Chloroprene	LH	2010	2B	1999	0.0005	M*	Mouse/ multiple	Limited
Acrylamide	LH	2010	2A	1994	0.0001	M*	Rat/ thyroid	Limited
Polychlorinated biphenyls	B2	1996	2A	2013	0.0001		Rat/liver	Very limited
1,3-Butadiene	CH	2002	1	2012	0.00003		Human/ leukemia	Strong (high exposures)
Formal dehyde	B1		1		0.000013		Human/nas al	Moderate (high exposures)
Vinyl chloride	CH	2010 Draft	1	2012	0.0000088		Rat/liver	Moderate (high exposures)
Benzene	CH	2003	1	2012	0.0000022 to 0.0000078		Human/ leukemia	Strong (high exposures)
Trichloroethylene	CH	2011	2A	2014	0.0000041	M*	Human/ kidney	Moderate
Epichl orohydrin	B2	1988	2A	1999	0.0000012		Rat/ kidney	Very limited
Tetrachloroethene	LH	2012	2A	2014	0.00000026		Mouse/ liver	Limited for bladder/NHL/ MM

US EPA WOE (2005 Guidelines) = CH - carcinogenic to humans; LH - likely to be carcinogenic; US EPA WOE (1986 Guidelines): A - human carcinogen; B1 - probable carcinogen, limited human evidence; B2 - probable carcinogen, sufficient evidence in animals; IARC WOE for carcinogenicity in humans (1 - carcinogenic; 2A - probably carcinogenic; 2B - possibly carcinogenic).; US EPA MOA (2005 Guidelines) M* - mutagenic and early life data lacking. NHL- non-Hodgkin lymphoma; MM – multiple myeloma

Despite being classified by IARC as a 2B carcinogen, chloroprene has the 5th highest IUR (see Table 8.1), which is orders of magnitude greater than the IURs for the known carcinogens vinyl chloride, 1,3-butadiene, and benzene. Three of the compounds with IURs higher than chloroprene (benzidine, bis(chloromethyl)ether [BCME], and N-Nitrosodimethylamine [NDMA]) have IURs that are based on reviews from the 1980s, performed before new methods were developed for integration of evidence, and likely would be different using current methods. Although there may be more recent data available to update the estimates for these compounds, two of these compounds are no longer of concern for human exposures: benzidine is no longer produced in the US (US EPA 1987a); additionally, there is very limited production of BCME, and what is produced or used is highly regulated (Bruske-Hohfeld 2009).

The only other compound with a higher IUR than chloroprene is ethylene dibromide (EDB)(US EPA 2004). US EPA (2004) described a single epidemiological st udy of occupational exposures to EDB, which was determined to be inadequate due to lack of exposure information and potential co-exposures to other carcinogens. Therefore, the IUR for ethylene dibromide was based on animal study results. Like

chloroprene, however, there were several important areas of uncertainty, including the extrapolation to low doses from high doses in rats, the application of the dose for respiratory tumors, portal of entry vs. systemic effects, and the need to account for metabolic differences between mice and humans. At the time of the assessment, a pharmacokinetic model was available (Hissink *et al*. 2000, Ploemen *et al*. 1995) but, as in the case of chloroprene, it was not deemed adequate for use by US EPA due to limited validation of the model. Therefore, updating the IUR for EDB also may be warranted. [8]

In contrast, there are several examples of carcinogenic compounds that have IURs that are *1 to 2 orders of magnitude lower* than chloroprene and for which US EPA has based the WOE evaluation and IUR development on much stronger positive human epidemiological evidence (1,3-butadiene and benzene) or for which US EPA appropriately used PBPK modeling to extrapolate results from animals to humans (vinyl chloride). In fact, one of the reasons US EPA classified chloroprene as a likely human carcinogen was structural similarities with 1,3-butadiene and vinyl chloride (US EPA 2010a), and it is particularly relevant to recognize how much higher the 2010 chloroprene IUR is compared to vinyl chloride and 1,3-butadiene. Both of these compounds were classified as known human carcinogens based on both stronger epidemiological evidence and supporting animal evidence than that available for cholorprene.

Vinyl chloride presents a relevant comparison to chloroprene based on its structural similarity to chloroprene and has been classified by IARC (2012) and US EPA (2000) as a known human carcinogen. Unlike chloroprene, however, the epidemiological evidence linking vinyl chloride with angiosarcomas of the liver, as well as primary hepatocellular cancers, is clear and consistent (Mundt *et al*. 2000, Boffetta *et al*. 2003, Mundt *et al*. 2017). US EPA appropriately applied a PBPK model for vinyl chloride to account for differences between animals and humans, resulting in a cancer IUR that is approximately 57 times lower than the IUR for chloroprene. When accounting for metabolic differences between animals and humans using a PBPK model, the cancer IUR for vinyl chloride was found to be consistent with risk estimates based on human epidemiological data and were lower than those based on external dose concentrations by a factor of 80 (Clewell *et al.* 2001).

1,3-butadiene has an extensive literature that describes its pharmacokinetics (US EPA 2002). Like chloroprene, the carcinogenetic mode of action of 1,3-butadiene is proposed to be related to its reactive metabolites, and results from PBPK models have demonstrated that there are important species differences in the rates of formation and detoxification of these reactive metabolites. In fact, the model results showed that, like chloroprene, pharmacokinetics can explain why mice are considerably more sensitive to the carcinogenic effects of 1,3-butadiene than other species, including humans. In comparing chloroprene with 1,3-butadiene, US EPA should have considered the differences observed across species that were also related to pharmacokinetics of 1,3-butadiene in deriving a chloroprene IUR, as similar differences across species have been observed for 1,3-butadiene.

[8] This is presented as a comparison for chloroprene, and is outside of the scope of our analysis.

There are other examples of recent assessments, such as that for trichloroethylene, for which US EPA appropriately applied a PBPK model to develop the IUR and for which epidemiological evidence is more robust than for chloroprene.

In summary, the comparison of the chloroprene IUR with the IURs of similar chemicals suggests that the chloroprene IUR from the 2010 Review is high even by IRIS standards, and that the chloroprene IUR should be reviewed and corrected.

9 A PBPK MODEL FOR CHLOROPRENE

9.1 PBPK modeling should be used to quantify the pharmacokinetic differences between species

PBPK modeling is used to predict the absorption, distribution, metabolism and excretion of chemical substances in humans and other animal species. These models are based on the integration of the available science for a specific compound. PBPK modeling is particularly important for use in extrapolating results from animal studies to develop toxicity values for humans, especially when there are significant differences across species. The "Guidelines for Carcinogenic Risk Assessment" (US EPA 2005) and the NRC review of the IRIS process (NRC 2014) recommend that if sufficient and relevant quantitative information is available (such as blood/tissue partition coefficients and pertinent physiological parameters for the species of interest), PBPK models should be constructed to assist in the determination of tissue dosimetry, species-to-species extrapolation of dose, and route-to-route extrapolation.

In the 2010 Review, US EPA acknowledged the shortcomings in their derivation of the chloroprene IUR, noting that: "Ideally, a PBPK model for the internal dose(s) of the reactive metabolite(s) would decrease some of the quantitative uncertainty in interspecies extrapolation; however, current PBPK models are inadequate for this purpose" (US EPA, 2010a). Although the PBPK models have been validated since the release of the 2010 Review, a PBPK model for chloroprene was available at the time US EPA prepared the 2010 Review. Despite uncertainties in the application of this model at the time of the development of the IUR, the results from these PBPK models would have explained the large observed inconsistencies in the data between mice, rats and humans. Additionally, there was substantial evidence at that time showing that external exposure concentrations from mouse chamber experiments were not representative of human health risks.

The 2010 Review noted that pharmacokinetic information on the absorption, distribution, and *in vivo* metabolism and excretion of chloroprene and/or its metabolites was available primarily for animals, but not humans. Several *in vitro* studies focused on chloroprene metabolism in lung and liver tissue fractions from rat, mouse, hamster, and humans (Cottrell *et al*. 2001; Himmelstein *et al*. 2001a, b; Himmelstein *et al*. 2004a, b; Hurst and Ali 2007; Munter *et al.* 2003; Munter *et al*. 2007; Summer and Greim 1980). These studies indicated that chloroprene is metabolized via the CYP450 enzyme system to active metabolites that are thought to be associated with the carcinogenic MOA for chloroprene. As noted in the 2010 Review, although the metabolic profile for chloroprene is qualitatively similar across species, *in vitro* kinetic studies using tissues from rodents and humans suggest significant interspecies and tissue-specific differences that, if operative *in vivo*, could account for the species, strain, and sex differences observed in chloroprene-induced *in vivo* effects.

The available *in vitro* information on the metabolism of chloroprene (Cottrell *et al.* 2001, Himmelstein *et al*. 2001b, Himmelstein *et al*. 2004a) demonstrates significant quantitative differences across species in the production of the major metabolites of chloroprene, and in particular, in the production of the epoxide likely to be the

carcinogenic constituent. The results from the *in vitro* studies indicate that greater amounts of these metabolites are produced in mice, followed by rats, and lastly in hamsters and humans. The 2010 Review discussed these differences, but did not incorporate this information when calculating the human equivalent dose for dose-response modeling. Himmelstein *et al*. (2004a) also noted species differences in the detoxification of epoxide metabolites, most notably the epoxide hydrolase, which serves to eliminate any epoxide formed. For example, the cross-species ranking of intrinsic clearance in the liver for enzymatic hydrolysis of the chloroprene metabolite was human ~ hamster > rat > mouse. In the lung, the order was human ~ hamster > rat ~ mouse. Therefore, the mouse not only had the highest capability for the generation of epoxide metabolites, but also the slowest capacity for clearance.

Overall, the balance of reactive metabolite formation and detoxification across species indicates that the mouse would be the most sensitive species, based on higher rates of epoxide formation, slower hydrolysis, and more enzyme activity. The mouse-specific pharmacokinetics all contribute to potentially increased formation and sustained concentrations of potentially toxic metabolites at lower exposures to chloroprene, explaining the increased sensitivity of this species.

The 2010 Review relied on the animal chamber air concentrations for the mouse exposure data to calculate the human IUR. Himmelstein *et al*. (2004b) demonstrated that there was no dose-response relationship when air concentrations from animal chambers (the administered dose) were used, whereas when the internal dose [9] was used (obtained from the PBPK model) a dose-response was clearly observed with relation to lung tumors. This is shown in Table 9.1, where the lung tumor incidence risk is assessed based on the internal dose. This table not only illustrates the dose-response based on internal dose, but clearly highlights the differences across species, showing that the mouse is the most sensitive species. When evaluating internal dose, which accounts for metabolic differences between mice, rats and hamsters, the differences in the lung tumor response across these species can be explained.

[9] In an experimental setting the administered dose is the concentration of the chemical that is given to the animal (measured in air, water, etc.), whereas the internal dose is the concentration of the chemical that is actually absorbed by the animal (measured inside the animal's body) and delivered to the target tissue.

Table 9.1. Exposure-Dose-Response for Rodent Lung Tumors

	Exposure concentration (ppm)	PBPK internal dose [a]	Lung tumor incidence	Number of animals	Extra risk (%)[b]
Hamster	0	0	0	100	0
	10	0.18	0	97	0
	50	0.88	0	97	0
Wistar rat	0	0	0	97	0
	10	0.18	0	13	0
	50	0.89	0	100	0
Fischer rat	0	0	3	50	0
	12.8	0.22	3	50	0.3
	32	0.55	6	49	7.7
	80	1.37	9	50	14.0
B6C3F1 mouse[d]	0	0	15	50	0
	12.8	3.46	32	50	48.3
	32	5.30	40	50	70.4
	80	7.18	46	50	89.9

(a) Internal dose - average daily mg Chloroprene metabolized/g lung tissue (AMPLU).
(b) The incidence data were corrected for extra risk equal to (Pi – Po)/(1 – Po), where P is the probability of tumor incidence in "i" exposed and "o" control animals (Himmelstein *et al*. 2004b).
(c) Male Syrian hamster and Wistar rat data from Trochimowicz *et al.* (1998).
(d) Male Fischer rat and B6C3F1 mouse data from Melnick *et al*. (1996).

9.2 US EPA calculation of the human equivalent concentration for chloroprene in the 2010 Review

All of the quantitative data necessary to refine and verify the critical metabolic parameters for the existing peer-reviewed PBPK model for chloroprene (Himmelstein *et al*. 2004b) were available at the time the 2010 Review was published and could have been applied to adjust the cancer unit risk to account for species-specific target-tissue dosimetry. Instead, the 2010 Review used the default approach and limited default assumptions described in the US EPA (1994) "Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry."

The 2010 Review assumptions included the following:

1. Lung tumors result primarily from systemic distribution, and
2. Chloroprene is a Category 3 gas according to US EPA (1994) guidelines.

Based on these assumptions, US EPA calculated the human equivalent concentration for chloroprene using the default DAF for Category 3 gases. As described by US EPA (1994), DAFs are ratios of animal to human physiologic parameters, and are based on the nature of the contaminant (particle or gas) and the target site (*e.g.*, respiratory tract) (US EPA 1994). For Category 3 gases with

systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients:

DAF = (Hb/g)A/(Hb/g)H

where:

(Hb/g)A = the animal blood:air partition coefficient

(Hb/g)H = the human blood:air partition coefficient

DAF = 7.8/4.5

DAF = 1.7

Furthermore, following US EPA guidelines (1994), US EPA used a default DAF of 1 because, as US EPA noted, "In cases where the animal blood:air partition coefficient is higher than the human value, resulting in a DAF>1, a default value of 1 is substituted (US EPA, 1994)." This was a conservative assumption, as it is noted in the guidelines that the available data for rats indicated that (Hb/g)A is greater than (Hb/g)H for most chemicals. This restricted the evaluation to equivalence between the mouse and the human and did not address the important pharmacokinetic differences in chloroprene metabolism in the mouse compared to the human.

9.3 The Allen et al. (2014) study shows that a validated PBPK model should be used to update the 2010 chloroprene IUR

Allen *et al.* (2014) combined the results from the most recent PBPK models for chloroprene (Yang *et al.* 2012) with a statistical maximum likelihood approach to test commonality of low-dose risk across species. Using this method, Allen *et al.* (2014) evaluated the difference between risk estimates obtained using external (chamber air concentrations) and internal dose (calculated with the PBPK model) metrics. The PBPK model for chloroprene incorporates data regarding species differences in metabolism of chloroprene, and allows species-specific estimation of internal exposure metrics, specifically the amount of chloroprene metabolized per gram of lung tissue. By using this model, IURs can then be compared across species based on equivalent internal exposure metrics rather than external air concentrations measured outside of the body. This is an important consideration when the toxicity of a compound is related to how the compound is metabolized in animals vs. humans.

Allen *et al.* (2014) found that for chloroprene, external concentration-based estimates were not appropriate for calculating and comparing cancer risks across species. As discussed in Section 5, epidemiological studies related to occupational exposures to chloroprene must also be considered in evaluating the unit risk estimate. These epidemiological studies provide little or no scientific support for the hypothesis that human and animal low-dose risks were equivalent when expressed as a function of air concentrations. In contrast, by accounting for the daily amount of chloroprene that is metabolized per gram of tissue at the target site for different species, the PBPK results provided a substantially better fit of the models to the data. Importantly, the differences in internal dose across species explained the greater sensitivity in mice (Himmelstein *et al.* 2004b), as well as the lower sensitivity of humans.

Allen *et al*. (2014) derived cancer unit risks for respiratory system cancer using the PBPK model results from both animal and human data that ranged from 2.9 x 10^{-5} to 1.4 x 10^{-2} per ppm (8.1 x 10^{-9} to 3.9 x 10^{-6} per µg/m^3), with a maximum-likelihood estimate of 6.7 x 10^{-3} per ppm (1.86 x 10^{-6} per µg/m^3). This estimate is about 100 times lower than the 2010 Review estimate of 6.5 x 10^{-1} per ppm (1.81 x 10^{-4} per µg/m^3) based on the incidence of lung tumors in female mice. It is also important to note that the Allen *et al*. (2014) assessment is highly conservative in that it does not account for species-to-species differences in detoxification and pharmacodynamics, which is justified and would lead to an even lower IUR.

It is difficult to apply the method used by US EPA for multi-tumor adjustment using the data provided in the Allen *et al*. (2014) publication, because the Allen *et al*. data were limited to lung tumors. However, this method likely would generate an estimate that is 100 times lower than the US EPA estimate. A similar rationale can be used for the application of the ADAF, yielding an IUR of approximately 5 x 10^{-6} per µg/m^3. However, because there is limited evidence for mutagenicity, we concluded that the 2010 IUR should be closer to the estimate calculated by Allen *et al*. (2014) of 1.86 x 10^{-6} per µg, and that this value is appropriately protective.

Overall, the evidence indicates that humans are far less sensitive to chloroprene exposures than mice, which is also consistent with the lack of clear or consistent epidemiological evidence of carcinogenicity as discussed in Section 5.

10 CALCULATION OF AN UPDATED CHLOROPRENE IUR

Ramboll Environ recalculated the IUR for chloroprene using the same standard methodologies that US EPA has employed in IRIS assessments for several known carcinogens, but did not employ in the 2010 Review of chloroprene. Ramboll Environ employed this methodology to reduce the significant uncertainty associated with extrapolating results from animal experiments to humans (and from one route of exposure to another), and in consideration of the substantial body of evidence demonstrating large differences in sensitivity to chloroprene across species. These differences reflect underlying pharmacokinetic differences that, if not taken into account, result in a highly inflated IUR value such as that derived in the 2010 Review.

The Allen *et al*. (2014) analysis provided a rigorous approach for integrating the available epidemiological and toxicological evidence to estimate a chloroprene IUR. However, it incorporated a maximum likelihood statistical method different from the traditional PBPK models used by US EPA in estimating IURs and other toxicity values, such as reference concentrations (RfC) or reference doses (RfD). In deriving an IUR, US EPA typically applies a PBPK model to estimate an internal dose at the target organ of interest (*e.g.*, the lung), based on the mode of action.

As discussed above, it is hypothesized that chloroprene itself does not exert a carcinogenic effect, but rather a metabolite of chloroprene exerts the effect. Therefore, carcinogenicity depends on the internal concentration of the metabolite, and not the internal (or external) concentration of chloroprene. The internal concentration of the metabolite is determined by how rapidly it is produced and eliminated from the body, and metabolite production and elimination rates vary considerably across species. Therefore, accounting for species-specific pharmacokinetic differences using PBPK modeling is critical. The US EPA (2005) Guidelines for Carcinogen Risk Assessment states that PBPK models

> *"…generally describe the relationship between exposure and measures of internal dose over time. More complex models can reflect sources of intrinsic variation, such as polymorphisms in metabolism and clearance rates. When a robust model is not available, or when the purpose of the assessment does not warrant developing a model, simpler approaches may be used."*

The preferred approach to PBPK modelling has been documented in the US EPA (2005) "Guidelines for Carcinogen Risk Assessment." Furthermore, US EPA has applied these PBPK models in estimating toxicity values for several compounds; for example, dichloromethane, vinyl chloride, tetrachloroethylene, carbon tetrachloride, and acrylamide, specifically to reduce uncertainty associated with animal-to-human extrapolation or route-to-route extrapolation. Although there may be no "perfect" model, toxicity values derived from models that best reduce uncertainty are more scientifically supportable and therefore preferred to those obtained using default adjustment factors (DeWoskin *et al*. 2007).

When an IUR is based on animal data, an animal PBPK model is required to estimate the internal dose corresponding to each of the administered

concentrations (*i.e.*, ppm in the chamber air), following the same pattern of exposure of the animals in the study (*e.g.*, days/week). This internal dose estimate is then used (instead of the air concentration) for dose-response modeling and estimating a Point of Departure (POD). This POD corresponds to the internal dose in the animal. The human PBPK model then is applied to account for known physiological and metabolic differences between the animal and human. This is accomplished by estimating the equivalent external concentration that results in the internal dose equal to the POD derived from the animal data. The IUR is estimated by dividing the risk level (benchmark risk or BMR associated with the POD) by the POD. The IUR is interpreted as the risk per unit (ppm or $\mu g/m^3$) intake.

Chloroprene PBPK modeling results for mice, rats, and humans are reported in Yang *et al*. (2012). Specifically, the internal dose estimates associated with the concentrations administered to both mice and rats in the NTP (1998) study are provided, including gender-specific internal tissues doses, *i.e.*, the average amount of chloroprene metabolized per day per gram of lung (AMPLU) based on the PBPK model. These internal doses represent the concentration of the toxic moiety (*i.e.*, the chloroprene metabolite) identified by US EPA as the key carcinogenic metabolite (US EPA, 2010a). The Yang *et al*. (2012) analysis showed that mice had the greatest amount of chloroprene metabolized per gram of lung, followed by rats and then humans. The human and rat showed linear dose-responses over the range of NTP bioassay concentrations of 12.8, 32 and 80 ppm. Based on this, the following was established as the relationship between the internal dose and the external exposure (ppm) in the human: 1 ppm of constant external exposure in the human results in 0.008 µmole of chloroprene metabolized per gram of lung tissue per day.

We relied on the internal dose results from the PBPK modeling conducted and reported by Yang *et al*. (2012), consistent with the PBPK modeling approach that US EPA has used in other IRIS assessments (dichloromethane, vinyl chloride, tetrachloroethylene, carbon tetrachloride). In addition, also consistent with the conclusions in the US EPA (2010) chloroprene review regarding the most sensitive endpoint in the most sensitive species, we estimated the chloroprene IUR using the results for the combined incidence of alveolar/bronchiolar adenomas and carcinomas (the most sensitive endpoint) in female mice (the most sensitive species and gender).

Using the internal doses for female mice as provided in Table 5 of Yang *et al.* (2012) (see Table 10.1), time-to-tumor modeling of the lung alveolar/bronchiolar adenomas and carcinomas was performed using the Multistage-Weibull model provided with the US EPA BMDS software (February 25, 2010 version). Time-to-tumor dose-response modeling is preferred and was used in the US EPA (2010) chloroprene assessment to model the incidence of tumors from the NTP (1998) bioassay. This type of dose-response model was necessary, as the survival of the female mice exposed to chloroprene was "significantly less than that of the chamber control" (NTP 1998). Time-to-tumor models adjust for early death of the animal, and thus the probability that the animal, if it had lived longer, may have developed the tumor of interest.

The female mouse data that we used in our analyses are presented in Table 10.2, with each animal's time of death and the observation of C, I, F or U to indicate: C=censored or the animal did not have the tumor of interest; I = incidental or the animal had the tumor of interest but it was not indicated as the cause of death; F=fatal or the animal had the tumor of interest and it was indicated as the cause of death; or U=unknown or the presence of the tumor could not be determined as the organ was autolyzed or missing in the animal. The alveolar/bronchiolar adenomas or carcinomas were all considered to be incident tumors, consistent with the time-to-tumor dose-response models and approaches used in US EPA (2010). One tumor was classified as unknown in one animal in the 12.8 ppm group, so modeling was conducted both including and excluding that animal to determine if there was any major impact on the outcome of the dose-response modeling.

Consistent with the US EPA (2010) approach, we selected a benchmark risk (BMR) of 1% (see Table 10.3 and Appendix C for the complete Multistage-Weibull modeling results). Note that models including or excluding the animal with the unknown tumor (Animal # 320)[10] generated the same estimated IUR. We calculated the external human dose (in ppm) by dividing the POD or lower bound on the benchmark dose (BMDL) by the factor of 0.008 to obtain the external concentration for continuous exposure in the human in ppm associated with the internal POD. We then calculated t he IUR by dividing the BMR by the human equivalent POD/BMDL in either ppm or µg/m^3:

□ □ = □ □/□ □ □ □ □ □

The final results are presented in Table 10 .4. Using the standard methods applied in other IRIS assessment by US EPA and publically available publish ed data, the recalculated IUR for chloroprene was 1.1x 10^{-2} per ppm or 3.2 x 10^{-6} per µg/m^3. This result, which incorporates appropriate PBPK models and adjustments necessary to extrapolate the findings from animal studies to relevant human exposure considering the differences in pharmacokinetics , is consistent with methods used in other IRIS assessments by US EPA. However, the IUR value is very different from that recommended in the 2010 Review and underscores the scientific importance of correcting and updating it.

[10] When it cannot be determined if an animal had the tumor of interest due to the organ being missing or deteriorated too much to examine, the animal will get an observation of "unknown". This data can be used in a time -to-tumor model (*e.g.* Multistage Weibull) as a time of death is available for that animal. In this case, including the animal with an observation of unknown or excluding the animal from the modeling did not result in a detectable difference in the results.

Table 10.1. Internal and External Doses from Yang et al. (2012)

External Dose (ppm)	PBPK Internal Dose Metric[11] (µmole CD metabolized /gram lung tissue/day)		Linear Relationship between ppm and PBPK metric in humans
	Mouse	Human	
12.8	0.74	0.1	0.008
32	1.19	0.25	0.008
80	1.58	0.64	0.008

[11] Data from Yang *et al.* (2012) Table 5.

Table 10.2. NTP (1998) Study – Female B6C3F 1 Mice Lung Alveolar/bronchiolar adenoma or carcinoma

Control = 0 ppm			Dose = 12.8 ppm			Dose=32 ppm			Dose = 80 ppm		
0 µmole/g tissue/day			0.74 µmole/g tissue/day			1.19 µmole/g tissue/day			1.58 µmole/g tissue/day		
Animal #	Time (wks)	Obs. [12]	Animal #	Time (wks)	Obs.	Animal #	Time (wks)	Obs.	Animal #	Time (wks)	Obs.
141	5	C	318	41	C	505	31	C	738	1	C
110	69	C	330	46	C	532	50	I	711	36	C
138	70	C	350	46	U	545	54	C	725	47	I
107	71	C	311	63	C	535	56	C	734	48	C
130	76	C	321	64	I	540	57	C	729	55	C
135	78	C	342	69	C	530	61	C	721	64	C
126	88	C	303	75	I	502	63	I	705	65	I
105	91	C	327	76	C	548	65	I	741	66	I
146	91	C	344	78	C	510	67	C	701	67	C
124	95	C	315	79	C	529	68	C	716	67	I
133	97	C	316	79	C	521	70	C	735	70	I
103	98	C	328	79	C	506	72	I	709	75	I
127	101	C	301	87	C	512	72	I	717	75	I
132	101	I	324	89	I	524	73	C	722	75	I
101	105	C	347	89	I	523	74	I	749	75	I
102	105	C	304	90	C	531	75	I	715	76	I
104	105	C	325	91	I	547	75	C	726	76	I
106	105	C	343	91	I	518	76	I	745	77	C
108	105	C	349	91	C	519	76	I	740	79	I
109	105	C	313	97	C	503	77	C	710	81	I
111	105	C	314	97	I	504	77	I	702	83	I
112	105	C	329	97	I	511	78	C	704	83	I
113	105	C	310	98	I	528	79	I	746	83	I
114	105	C	308	99	C	546	79	I	714	84	I
115	105	C	319	99	I	533	82	I	730	86	I
116	105	C	323	99	I	520	84	I	703	87	C
117	105	C	332	99	I	522	84	C	713	88	I
118	105	C	340	99	I	536	86	I	728	88	I
119	105	C	345	100	C	507	87	I	712	90	I
120	105	C	306	101	I	525	87	C	737	90	I

[12] Observation s are coded as C=censored, the animal did not have the tumor of interest
I = Incidental, the animal had the tumor of interest but it did not cause death
F = fatal, the animal had the tumor of interest and it was the cause of death (none in this dataset)
U = Unknown, it is not known if the animal had the tumor or not due to organ being autolyzed or missing

Control = 0 ppm			Dose = 12.8 ppm			Dose=32 ppm			Dose = 80 ppm		
0 µmole/g tissue/day			0.74 µmole/g tissue/day			1.19 µmole/g tissue/day			1.58 µmole/g tissue/day		
Animal #	Time (wks)	Obs.[12]	Animal #	Time (wks)	Obs.	Animal #	Time (wks)	Obs.	Animal #	Time (wks)	Obs.
121	105	C	334	102	I	526	87	I	718	91	I
122	105	C	346	102	I	527	89	I	727	91	I
123	105	I	331	103	C	539	89	I	732	91	I
125	105	C	341	103	I	541	90	I	733	91	I
128	105	C	302	105	I	542	90	I	736	91	I
129	105	C	305	105	I	544	90	I	747	91	I
131	105	I	307	105	I	501	91	I	750	91	I
134	105	I	309	105	C	509	91	I	724	92	I
136	105	C	312	105	C	516	91	I	742	93	I
137	105	C	317	105	I	537	92	I	748	93	I
139	105	C	320	105	I	508	93	I	707	94	I
140	105	C	322	105	I	517	94	I	708	95	I
142	105	C	326	105	C	538	94	I	739	95	I
143	105	C	333	105	C	550	94	I	744	96	I
144	105	C	335	105	I	534	96	I	723	97	I
145	105	C	336	105	I	549	96	C	731	97	I
147	105	C	337	105	I	513	97	I	743	98	I
148	105	C	338	105	C	515	99	C	706	105	I
149	105	C	339	105	I	543	103	I	719	105	I
150	105	C	348	105	I	514	105	I	720	105	I

Table 10.3. Multistage -Weibull Time-to-Tumor Modeling Results for a Benchmark Risk of 1%

Site	Stages	Log-Likelihood	AIC	Model Selection	BMD (µmole/gram lung tissue/day)	BMDL (µmole/gram lung tissue/day)	BMDU (µmole/gram lung tissue/day)
Female Mouse Lung – incidental. Animal with unknown status excluded	3	-82.607	175.21		0.0098	0.0052	0.0783
	2	-82.669	173.34	Lowest AIC	0.0677	0.0069	0.0770
	1	-85.722	177.44		0.0049	0.0039	0.0060
Female Mouse Lung – incidental. Animal with unknown status included	3	-82.674	175.35		0.0099	0.0053	0.0791
	2	-82.739	173.48	Lowest AIC	0.0676	0.0070	0.0768
	1	-85.882	177.77		0.0048	0.0037	0.0060

Table 10.4. Calculation of IURs using Human Equivalent Concentrations

Results from 2-stage Multistage Weibull Time-to-tumor model	BMR = 0.01				
	BMDL (µmole/gram lung tissue/day)	External Concentration (ppm) [13]	IUR (per ppm)	External Concentration (µg/m^3)	IUR (per µg/m3)
Female Mouse Lung – incidental. Animal with unknown status excluded	0.0069	0.863	0.012	3122	3.2E-06
Female Mouse Lung – incidental. Animal with unknown status included	0.0070	0.875	0.011	3168	3.2E-06

[13] Human doses in ppm are obtained by dividing the BMDL by the conversion factor derived from Yang et al. (2012) Table 5 of 1 ppm = 0.008 µmole/gram lung tissue/day

11 CANCER RISK ASSESSMENT: VALIDATION OF THE CHLOROPRENE IUR

As a validity check, we calculated the excess cancers that would be expected based on application of the US EPA IUR at the chloroprene exposure concentrations reported by Marsh *et al*. (2007b). Marsh *et al*. (2007b) modeled the chloroprene exposures for all unique job title classes using six exposure classes for each plant over the entire period of chloroprene production in each plant. Job title classes and time-specific chloroprene exposure estimates were linked to each worker's job history to construct a profile. These subject-specific profiles were then used to compute the statistical estimates of worker exposures used in the risk calculations presented in Table 11.1.

As shown in Table 11.1, we calculated risk estimates (excess cancers) for each of the unit risk estimates that US EPA derived for chloroprene in the 2010 Review. These included an IUR based on lung tumors, an IUR based on multiple tumors, and an IUR adjusted for lifetime exposures (with application of the ADAF). In addition, we calculated cancer risk estimates based on the IUR derived by Allen *et al*. (2014), as well as the IUR provided in this report, both of which account for pharmacokinetic differences between animals and humans. We derived risk estimates using exposure estimates from the Louisville plant (Marsh 2007a, b), as these exposures were much higher (at least an order of magnitude or more) than the exposures at other plants. In Table 11.1, we compared calculated excess cancer risk estimates with the excess liver cancers observed at the Louisville plant (observed cases minus expected cases, based on both US and local county rates).

The risk assessment summarized in Table 11.1 illustrates that cancer risk estimates calculated based on the IUR in the 2010 Review overestimated actual liver cancer risks. Marsh *et al*. (2007a) reported less than one excess liver cancer death when compared to US rates, and a deficit of about two liver cancer deaths when compared to the more appropriate local country rates. In contrast, using the 2010 Review IUR and mean reported chloroprene exposures, approximately 15 excess liver cancer deaths should have been observed. Repeating this exercise using the risk estimate derived by Allen *et al*. (2014), as well as the Ramboll Environ estimated IUR in this report, we showed that the estimated excess cancer risk estimates were consistent with the observed cases reported by Marsh *et al*. (2007a).

Table 11.1. Cancer Risk Estimates Based on US EPA and Allen et al. (2014) IURs for Chloroprene Compared with Excess Cancers Observed in the Louisville Plant

Source	Unit risk (per ppm)	Exposure (ppm)[a]			Excess Cancers (Risk Estimate)[b]			Excess Liver Cancers (Observed-Expected)[c]	
								Comparison Group	
		Median	Mean	Max	Median	Mean	Max	US	Local County
US EPA (2010)									
lung tumor	0.65	5.23	8.42	71	3.40	5.5	46		
multi tumor	1.08	5.23	8.42	71	5.65	9.1	77		
w/ADAF	1.80	5.23	8.42	71	9.41	15.2	128	0.65	-1.89
Allen et al. (2014)									
lung tumor	0.0067	5.23	8.42	71	0.04	0.1	0.5		
Ramboll Environ									
lung tumor	0.011	5.23	8.42	71	0.06	0.1	0.8		

a Data from Marsh *et al*. 2007b (Table 3)
b Excess cancer risk calculated by multiplying the unit risk (per ppm) by the exposure level (in ppm)
c Data obtained from Marsh *et al*. 2007a (Table 3). Expected cancers = Observed/SMR

This analysis demonstrates that the 2010 Review IUR overestimates risk, and that a PBPK adjustment provides a better fit to the best available human data.

12 THE CHLOROPRENE RFC

A reference concentration (RfC) is a health risk value that is intended to be protective of non-cancer risks from inhalation in humans. The RfC reported in the 2010 Review for chloroprene is 2×10^{-2} mg/m^3. The RfC is an estimate of the daily exposure to human populations, including susceptible groups such as children and the elderly, which is considered to be without an appreciable risk for non-cancer health effects over a lifetime. The value is calculated by first determining the point of departure, traditionally using a no-observed-adverse-effect level or lowest-observed-adverse-effect level (NOAEL or LOAEL, respectively) and more recently using dose-response modeling.

Like the calculation of the cancer IUR, US EPA relied upon the results from the 2-year chronic inhalation study conducted in rats and mice by the National Toxicology Program (NTP 1998) as the basis for the RfC, but focusing on the non-cancer effects. US EPA also considered a second study conducted in a different strain of rats and in hamsters (Trochimowicz *et al.*, 1998), but did not rely on this study because it reported a high mortality rate in animals in the lowest exposure group due to failure in the exposure chamber. However, though significant histopathological lesions were reported in the NTP (1998) study in the lungs and spleen in the lowest exposure group (12.8 ppm) in B6C3F1 mice, comparatively few histopathological lesions were observed even in the highest exposure groups in Wistar rats and Syrian hamsters (Trochimowicz *et al.*, 1998).

From the NTP (1998) study, US EPA selected all the non-cancer endpoints that were statistically significantly increased in mice and rats at the low and mid-exposure levels (12.8 and 32 ppm) compared with controls. These endpoints included both portal of entry and systematic lesions observed in the nose, lung, kidney, forestomach, and spleen in mice and in the nose, lung and kidney of the rats (see Table 5-1 in US EPA 2010a). US EPA used their own benchmark dose modeling software (BMDS) to estimate a Point of Departure (POD). As with the cancer endpoints, these results suggested significant cross-species and strain differences in the toxicological response to inhaled chloroprene. In addition, for some of the endpoints, no model provided an adequate fit to the data, suggesting external concentrations may not correspond to the observed incidences. These results also underscore the importance of understanding the difference in pharmacokinetics across species to derive the most biologically relevant human equivalent RfC. PBPK methods have been used to derive appropriate RfCs for other relevant chemicals, including vinyl chloride (Clewell 2001, US EPA 2000).

The last source of uncertainty that US EPA should have considered in the derivation of the RfC is the application of uncertainty factors to the POD. US EPA applied a total uncertainty factor of 100 to the POD of 2 mg/m^3. A standard uncertainty factor of 10 was applied to account for variation in the susceptibility among members of the human population. An uncertainty of 3 was applied to account for extrapolation of animals to humans; however, this uncertainty can be removed if a validated PBPK model is used to derive a human equivalent exposure to chloroprene that accounts for pharmacokinetic differences between animals and humans. Lastly, an uncertainty factor of 3 was applied to account for database

deficiencies related to reproductive toxicity. This adjustment is also not needed based on several lines of evidence. First, chloroprene is not expected to accumulate in tissues such that in a multigenerational study, exposures to the second generation (F2) would be greater than experienced by the first generation (F1). Second, the results of a single generation reproductive toxicity study for a structurally similar chemical, 2,3-dichloro-1,3-butadiene (Mylchreest *et al*. 2006) indicate that effects at the point of contact (nasal effects) in parental animals are more sensitive than reproductive/developmental effects. Specifically, this study reported a NOAEL of 10 ppm for nasal effects in rats, and a NOAEL of 50 ppm for reproductive toxicity (changes in maternal and fetal body weights). Similarly, an unpublished one-generation reproductive toxicity study of chloroprene in rats reported a NOAEL of 100 ppm for reproductive toxicity (Appelman and Dreef van der Meulan 1979). All of these NOAELs are considerably higher than any other non-cancer effect and suggest that the application of an uncertainty factor for database deficiencies for the lack of a two-generation reproductive study is not necessary.

13 CONCLUSIONS

The IUR derived in the 2010 Report did not address the large recognized differences in cancer susceptibility across animal species, and especially between female mice and humans. Failure to apply well-accepted and now specifically validated methods for accounting for these differences led to an invalid (and implausible) IUR for chloroprene.

Our critical review and synthesis of the available evidence from toxicological, mechanistic, and epidemiological studies, as well as an integration of the evidence across these lines of scientific inquiry, determined that the approach US EPA used to derive an IUR for chloroprene relied on several unsubstantiated assumptions and failed to take into account the large inter-species cancer susceptibilities. We demonstrated that an IUR derived today would be considerably different from the one recommended in the 2010 Review. Our approach comported with US EPA methods and guidance, as well as the recommendations made by multiple NRC Committees evaluating the US EPA IRIS evaluation methods.

Although animal studies provided a positive response for carcinogenicity, the current science for chloroprene demonstrates major differences in species-specific cancer response to chloroprene exposure. Quantitative differences in pharmacokinetics across species, specifically related to differences in metabolism and detoxification of potentially active metabolites, can and should be incorporated into a corrected IUR or other risk number. In the 2010 Review, the available chloroprene pharmacokinetic findings were not incorporated to quantitatively account for differences between the mouse, rat, and human. When genotoxicity/genomics, MOA, and pharmacokinetic data are considered in an appropriately integrated manner, the data strongly suggest that the cancer responses from chloroprene are largely confined to—and possibly unique to—the female mouse. Because of these strong interspecies differences, use of the female mouse data for risk evaluation, in the absence of affirmative epidemiologic al data that can be used quantitatively, must incorporate tissue-specific dosimetry and metabolic differences. Additionally, because the available evidence does not support a mutagenic MOA for chloroprene, the cancer unit risk should not be adjusted to account for potential risks from early-life exposures with the application of the ADAF. While appropriate PBPK models were available to US EPA at the time of the 2010 Review, US EPA stated that published data were unavailable to validate the model. Data have now been published, have validated the PBPK model, and should be used to correct the IUR.

Our critical review and synthesis of all epidemiological studies of chloroprene-exposed workers, using standard methods that consider study quality and potential sources of bias, indicated no clear or consistent association between occupational chloroprene exposure and mortality from lung or liver cancers. The strongest study, in fact, demonstrated small deficits in lung and liver cancer mortality among chloroprene-exposed workers (Marsh 2007a, b). Nevertheless, in the 2010 Review, this study is cited as providing support for a causal association, directly contradicting our conclusions as well as the study authors' own conclusions. In fact, the epidemiology was consistent with the application of a PBPK model to

adjust the animal experimental evidence and account for the large differences in inter species cancer susceptibilities. There is a substantial body of evidence supporting the conclusion that humans are far less susceptible to the potential carcinogenicity of chlor oprene than mice primarily because the way humans metabolize chloroprene does not lead to the production of significant concentrations of the carcinogenic metabolite. The epidemiological study results also support this conclusion.

Using standard methods consistent with the NRC recommendations and EPA Guidelines, and the most current scientific evidence, we derived an IUR for chloroprene that is 156 times lower than that derived by US EPA. Following methods used in other IRIS assessments, we derived an IUR of 3.2×10^{-6} per $\mu g/m^3$. We request that US EPA re-evaluate and correct the IUR, which is based on the most sensitive species and endpoint (lung tumors in female mice) and apply a PBPK model to more appropriately account for the large differences between mice and humans. We recommend no further adjustment for multiple tumor sites, and no adjustment for a mutagenic MOA. Similarly, the chloroprene RfC will need to be updated to incorporate the same pharmacokinetic differences across species.

Based on a comprehensive evaluation and integration of the published epidemiological, toxicological and mechanistic evidence, we consider the US EPA 2010 Review of chloroprene to be outdated and invalid. Accordingly, US EPA should also revisit the cancer classification for chloroprene and provide a transparent and accurate narrative that reflects a weight of evidence approach. Most importantly, however, the IUR derived in the 2010 Report is not scientifically defensible and needs to be corrected.

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APPENDIX A
TOXICOLOGICAL SUMMARY OF CARCINOGENIC COMPOUNDS

Toxicological Summary of Carcinogenic Compounds

Chemical	IUR (per µg/m³)	US EPA WOE/ Year	Human Data	Animal Data	Geno-toxicity	Extrapolation Method	Species	Endpoint	Model Used	PBPK Model
Benzidine**	0.067	A/1987	Sufficient	Limited *via* inhalation	Yes	One-hit with time factor, extra risk	Human Occupational (Inhalation)	Bladder tumors	--	No
Bis(chloromethyl)ether (BCME) **	0.062	A/1988	Sufficient	Sufficient	Yes	Linearized multistage, extra risk	Rat	Respiratory tract tumors	--	No
N-Nitrosodimethylamine (NDMA **)	0.014	B2/1987	Limited due to exposure to mixtures	Limited evidence *via* inhalation	Yes	Weibull, extra risk	Rat	Liver tumors	--	No
Ethylene Dibromide	0.0006	B2/2004	Inadequate	Sufficient	Yes	Multistage	Rat	Nasal cavity tumors	Multistage-Weibull time-to-tumor	No
Chloroprene	0.0005	B1/2010	--	Clear evidence	Yes - Metabolites	Linear low-dose extrapolation	Mice	All tumor sites reported	Multistage-Weibull time-to-tumor	No
Acrylamide	0.000147	B2/2010	Inadequate	Sufficient	Yes	Route-to-route extrapolation of the oral POD	Rat	Thyroid tumors	Multistage-Weibull Time-to-tumor	No
Polychlorinated biphenyls (under reassessment)#	0.0001	B2/1996	Inadequate	Sufficient	--	Linear extrapolation below LED10s	Rat	Liver tumors	--	No
1,3-Butadiene	0.00003	A/2002	Sufficient	Sufficient	Yes - Metabolites	Linear extrapolation	Human	Leukemia	Relative Rate Model	No